National Institute of Allergy and Infectious Diseases

7th Annual Texas Medical Center Antimicrobial Resistance and Stewardship Conference

Probiotics for *Staphylococcus aureus*: A Translational Approach

January 18, 2024

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National Institute of Allergy and Infectious Diseases

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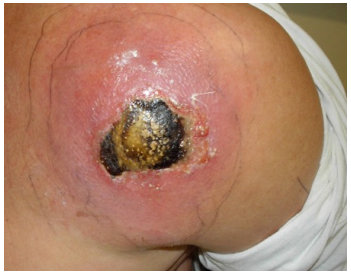
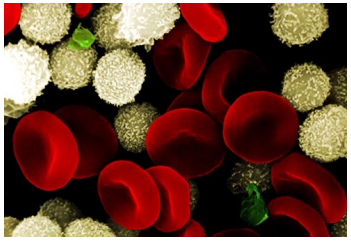
Laboratory of Bacteriology

Staphylococcus aureus infections

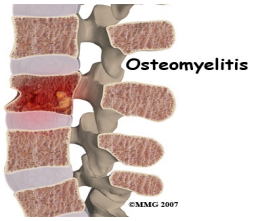
- Leading human and mammal pathogen
- Frequent antibiotic resistance (MRSA)
- Hospital-associated and community-associated infections



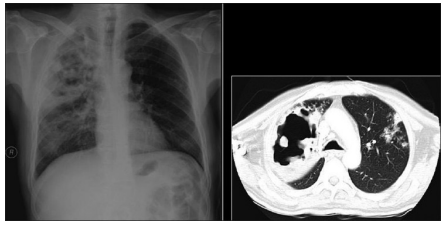
Abscess



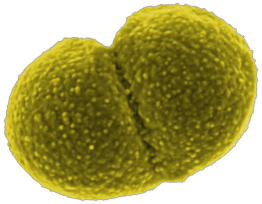
Sepsis

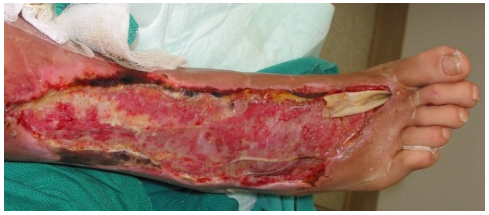


Osteomyelitis

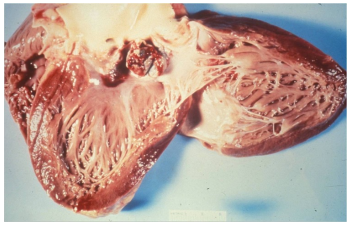


(Necrotizing) pneumonia

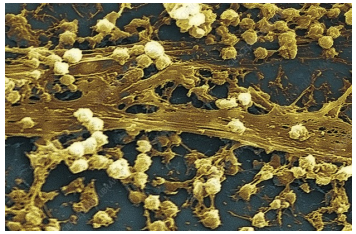




Necrotizing fasciitis



Endocarditis



Device infections

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S. aureus carriage

- Asymptomatic colonization in ~ 20-30% (throat, perineum, groin, anterior nares, skin)
- The nares have traditionally been considered the primary *S. aureus* colonization site, but the intestinal tract is also commonly colonized by *S. aureus*.
- *S. aureus* intestinal colonization is associated with nasal colonization and increased frequency of positive skin cultures.

Treatment alternatives for *S. aureus* in the age of antimicrobial resistance

- *S. aureus* is often (multi-)resistant to antibiotics.
- Methicillin resistance (MRSA) increases morbidity, mortality, cost and length of hospital stay.
- Possible alternatives to antibiotics include
  - Vaccines, mAbs (so far unsuccessful)
  - Anti-virulence strategies (quorum-sensing blockers etc.; pre-clinical)
  - Immune stimulation (pre-clinical)
  - Bacteriophages

Decolonization

- Decolonization as a means to reduce *S. aureus* infection rates is based on the fact that colonization increases the risk of infection.
- Shown for nasal colonization and nosocomial bacteremia (Van Eiff et al. *NEJM* 2001), but also rectal carriage and infections in an intensive care unit (Squier et al. *Infect Control Hosp Epidemiol* 2002).
- Problem: Use of topical antibiotics (mupirocin); fast recolonization after treatment (potential causes: recolonization from other body sites, other individuals).

Probiotics – Microbiome editing

Probiotics are foods or supplements that contain live microorganisms intended to maintain or improve the "good" bacteria (normal microflora) in the body.

However, underlying mechanisms are often unclear.
Effects usually broad (such as, immune-stimulatory)

”Precision microbiome editing” is the alteration of the microbiome in a very targeted fashion, allowing the eradication or suppression of microbes associated with specific diseases.

However, our knowledge about microbial interactions in the microbiome is still too limited for such approaches in most cases.
Bacteriocin-producing strains are often suggested, but – while more target-specific than antibiotics – bacteriocins still also kill a lot of “good” bacteria.

Overview

Mechanism:

Pathogen elimination by probiotic *Bacillus* via signalling interference

Pipat Piewngam[1,2], Yue Zheng[1,5], Thuan H. Nguyen[1,5], Seth W. Dickey[1], Hwang-Soo Joo[1,4], Amer E. Villaruz[1], Kyle A. Glose[1], Emilie L. Fisher[1], Rachelle L. Hunt[1], Barry Li[1], Janice Chiou[1], Sujiraphong Pharkjaksu[2], Sunisa Khongthong[3], Gordon Y. C. Cheung[1], Pattarachai Kiratisin[2] & Michael Otto[1]*

Nature 2018, 562, 532-537

Clinical trial/application:

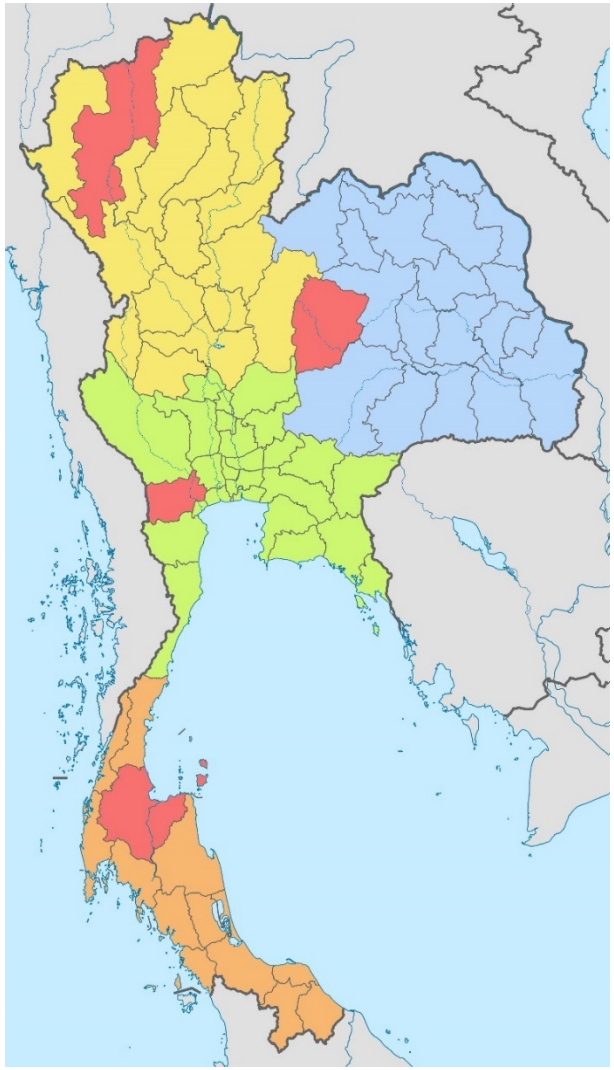
Probiotic for pathogen-specific *Staphylococcus aureus* decolonisation in Thailand: a phase 2, double-blind, randomised, placebo-controlled trial

Pipat Piewngam, Sunisa Khongthong*, Natthrit Roekngam, Yongyuth Theapparat, Somkiat Sunpaweravong, Damrongsak Faroongsarng, Michael Otto*

Lancet Microbe 2023, 4, e75-83

Study on nasal and fecal *S. aureus* carriage in rural Thailand

Pipat Piewngam



Healthy individuals (N=200)

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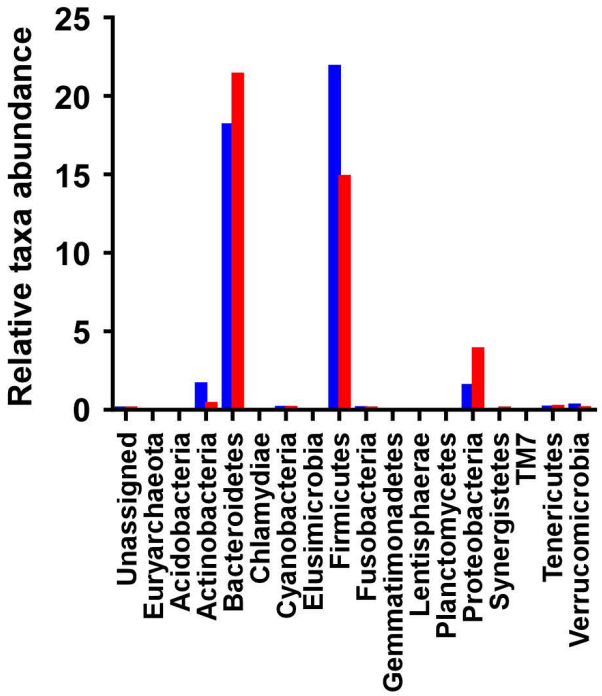
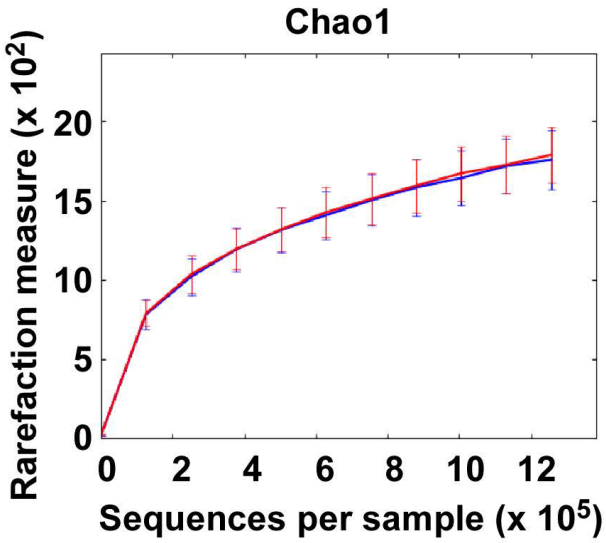
Comparison of microbiota profiles between *S. aureus* carriers and non-carriers





We did not detect significant differences in the composition of the microbiome between *S. aureus* carriers and non-carriers.

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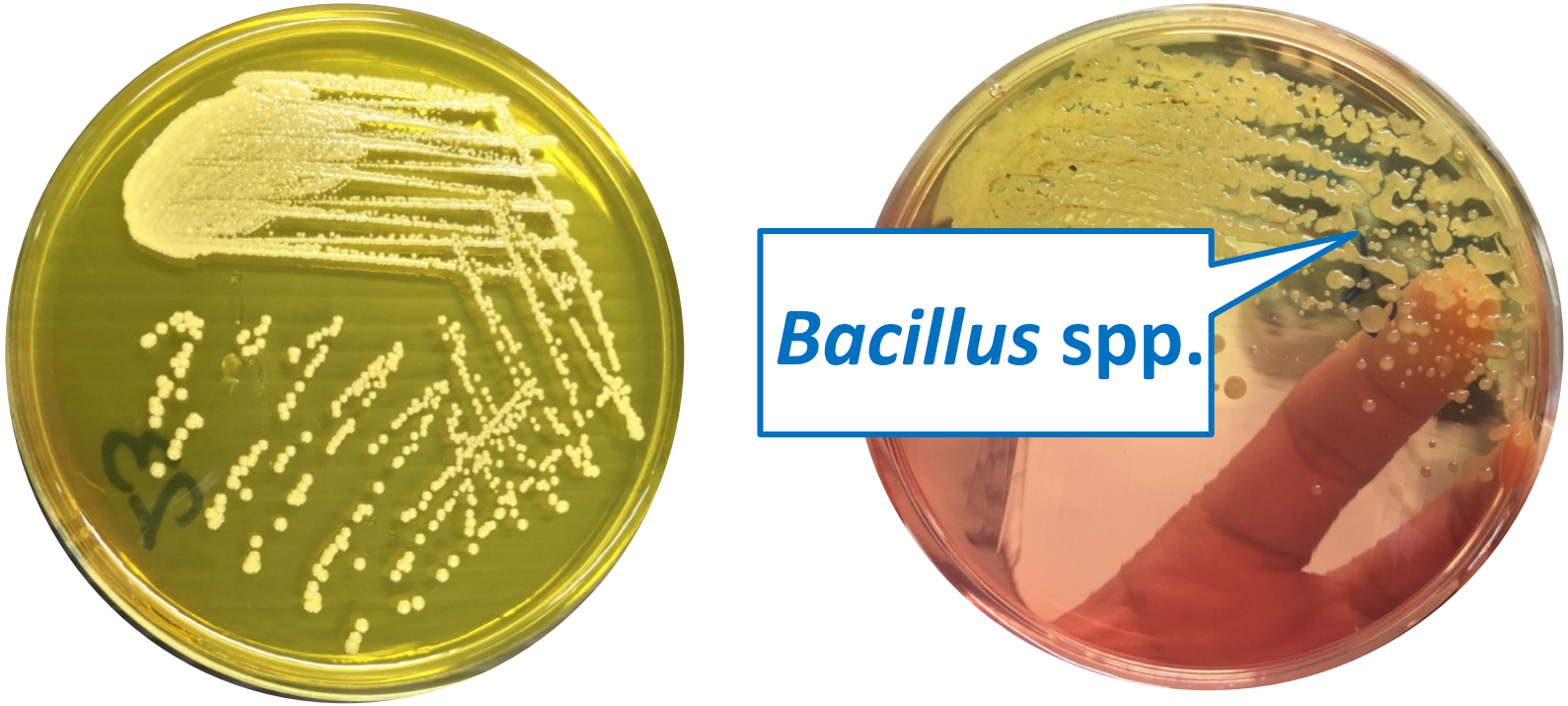
Fecal samples grow either *Bacillus* or *S. aureus*, but not both.



Mannitol Salt agar (MSA)
All isolates confirmed by MALDI-TOF

***S. aureus* carrier** ***S. aureus* non-carrier**

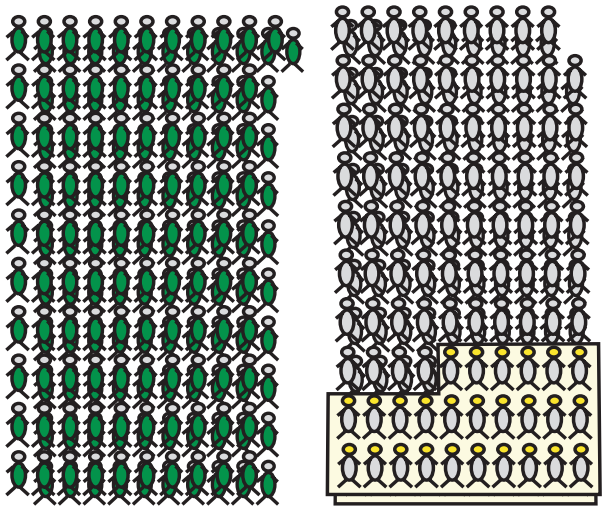
Bacillus spp. - *S. aureus* exclusion effect

Colonized by *Bacillus*

50.5%

Not colonized by *Bacillus*

49.5%

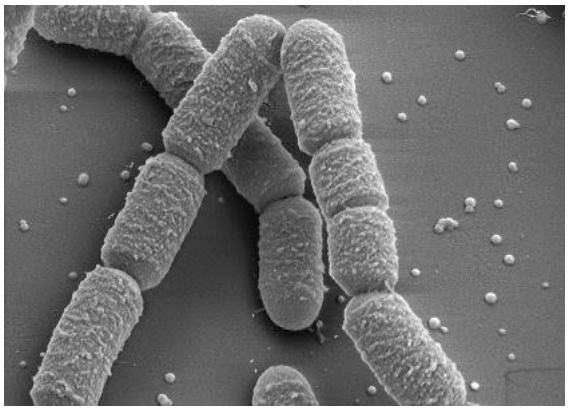


***S. aureus* nasal colonizers**

S. aureus colonization was only observed in the absence of *Bacillus*, indicating efficient *Bacillus*-mediated *S. aureus* colonization exclusion.

Bacillus spp.

- Gram-positive, catalase-positive bacterium.
- forms resistant spores.
- can be found in the soil and gastrointestinal tract of ruminants and humans. Spores shown to germinate in the gut.
- compound in over-the-counter probiotics (Probiotic activity poorly understood).

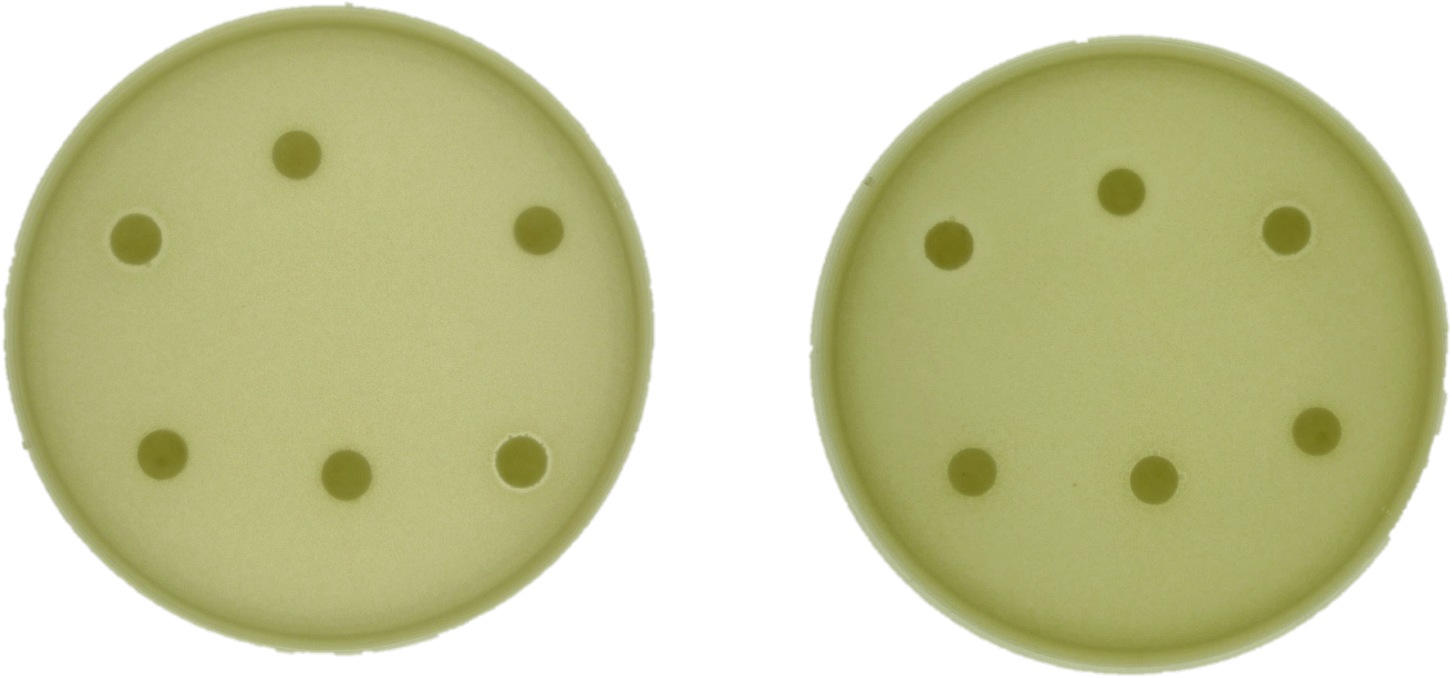


Hypothesis

***Bacillus* spp. produce substances that can inhibit *S. aureus* growth or interfere with *S. aureus* gut colonization.**

Not a bacteriocin…

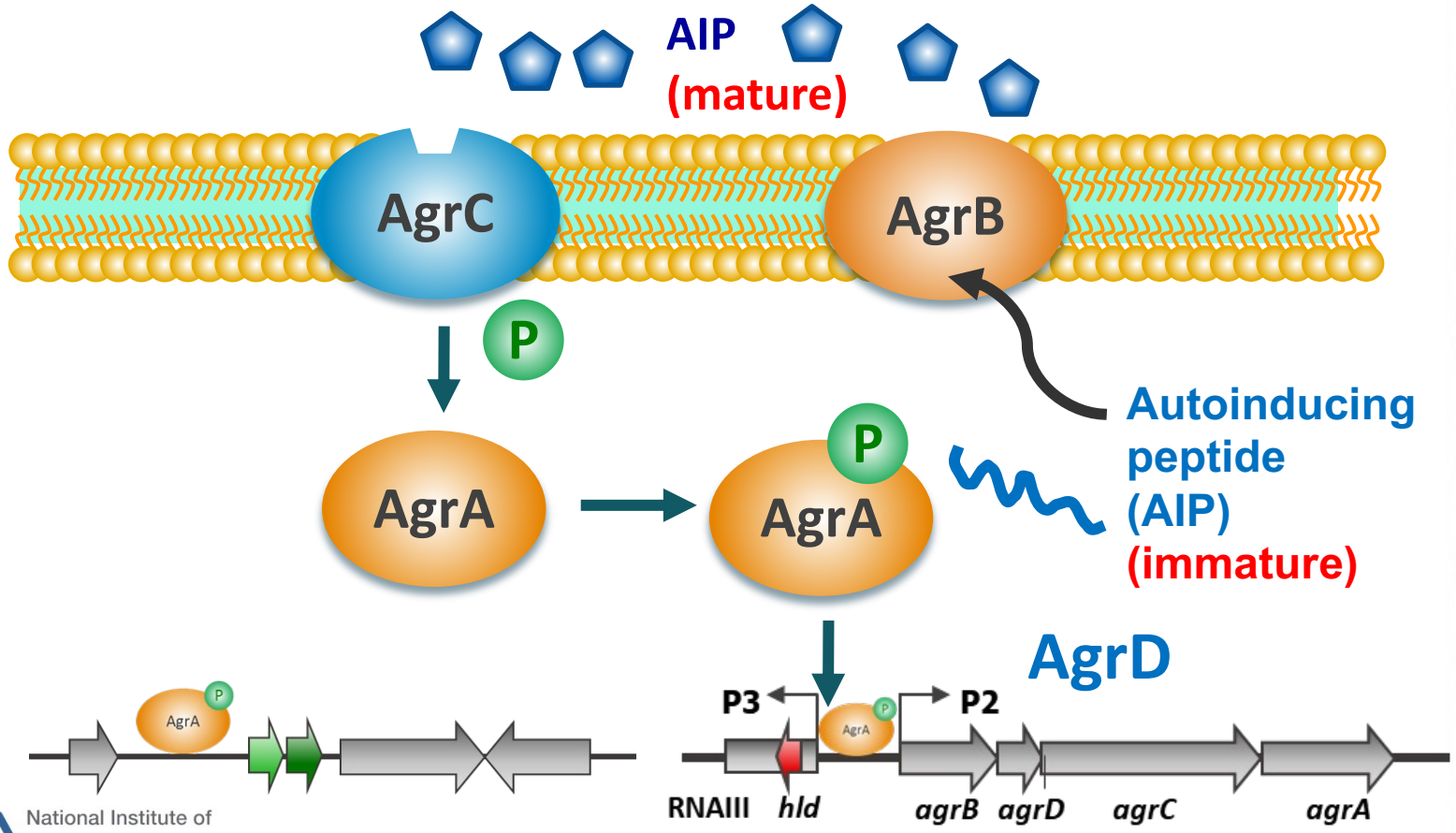
5x conc. culture filtrate



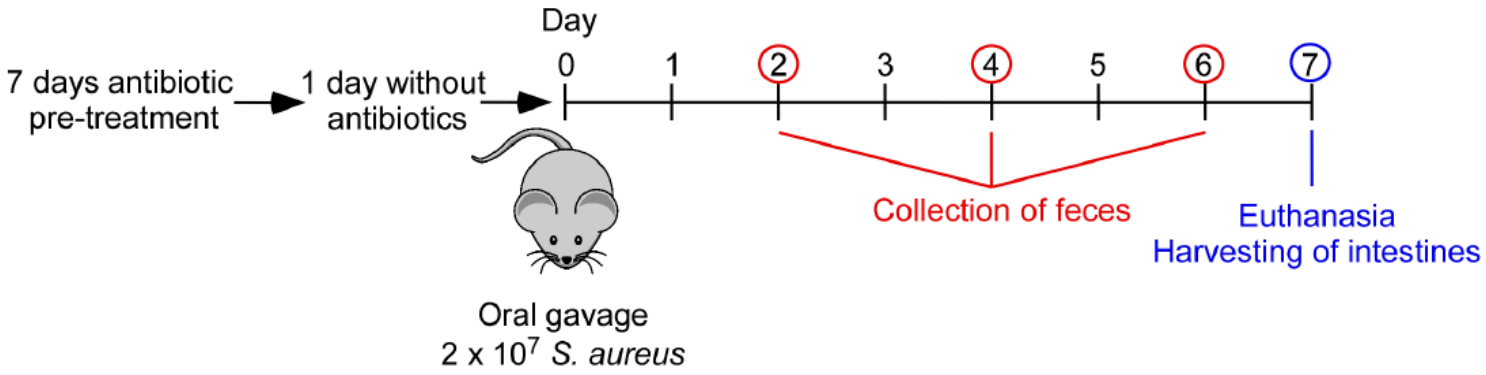
Only very minor growth-inhibitory effects were observed in 6/105 isolates.
=> Not the answer

Quorum-sensing?

QS in *S. aureus* (Agr system) regulates a plethora of virulence factors, but hasn't been implicated in colonization.



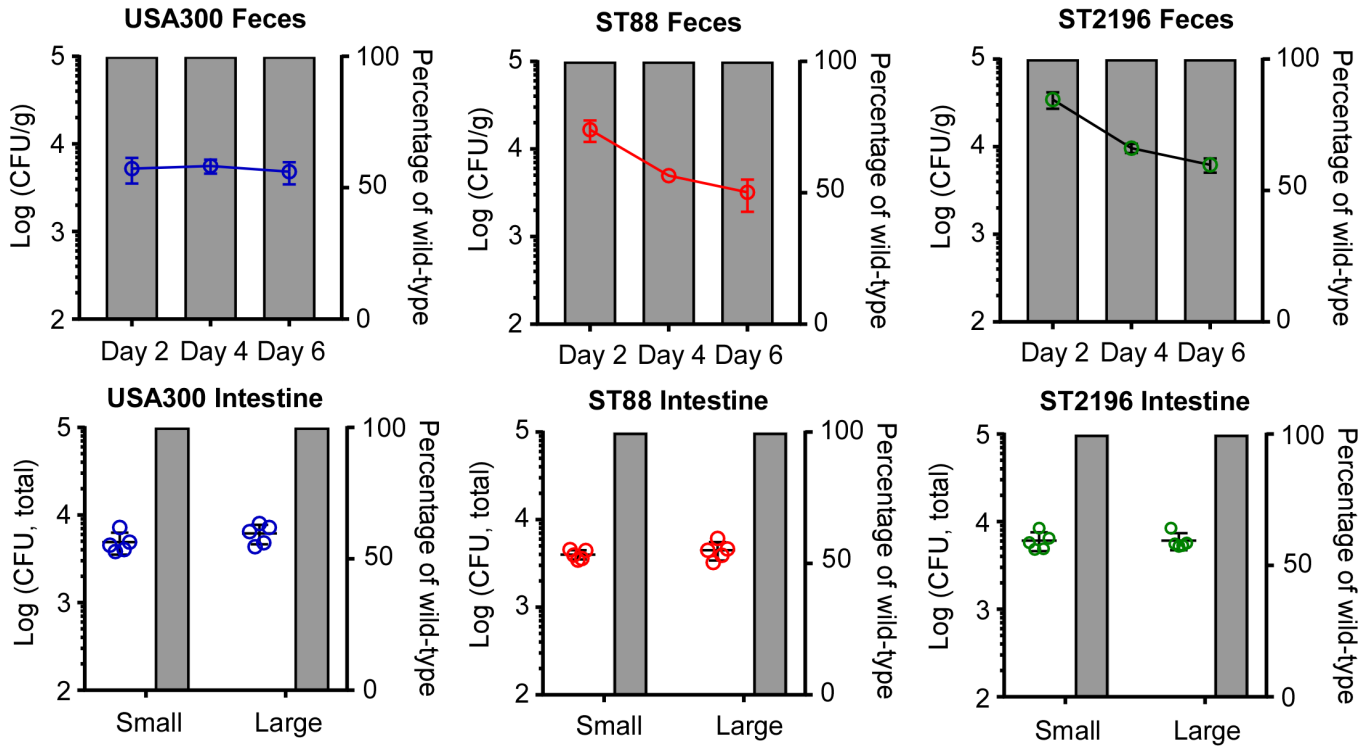
Role of Agr in intestinal colonization – mouse experiment setup



Bacterial strains

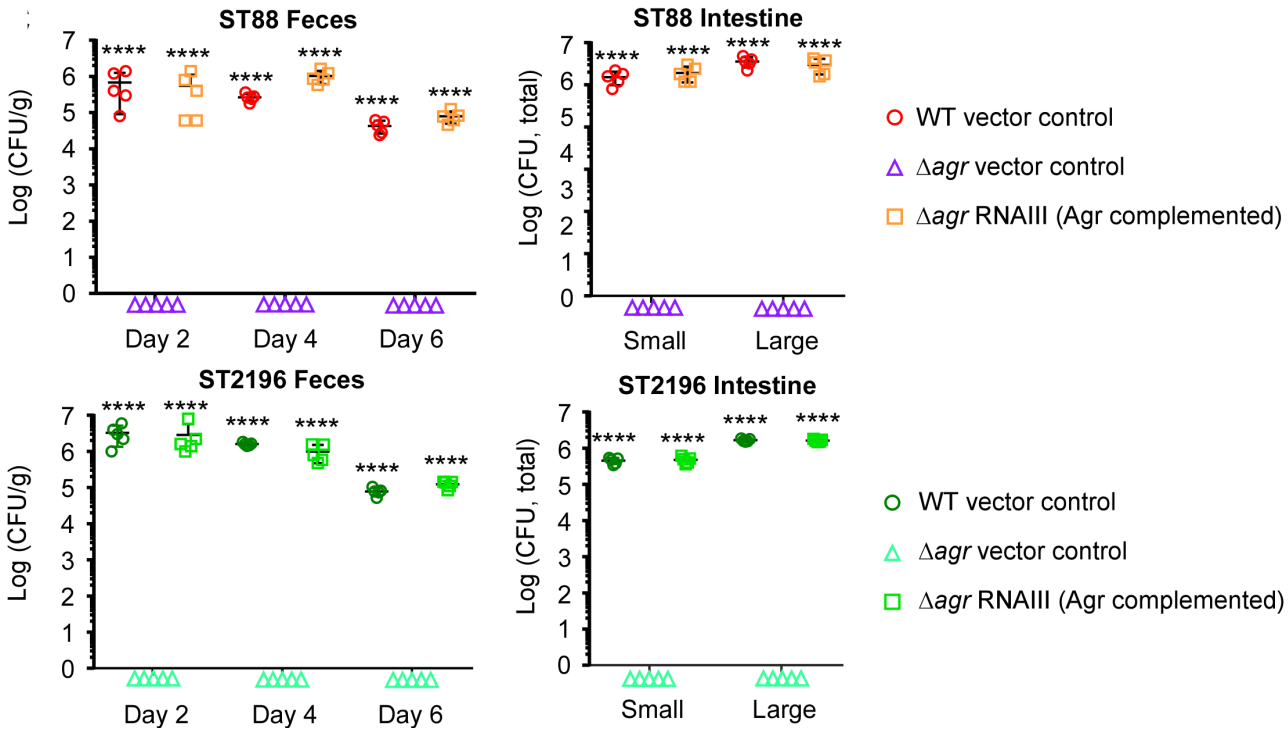
- USA300 (human infection isolate, CA-MRSA)
- ST88 (mouse-adapted strain)
- F12 (ST2196, human fecal isolate from our study)

Competition experiments with equal amounts of wild-type and isogenic *agr* mutant



S. aureus agr mutant is dramatically impaired in intestinal colonization.

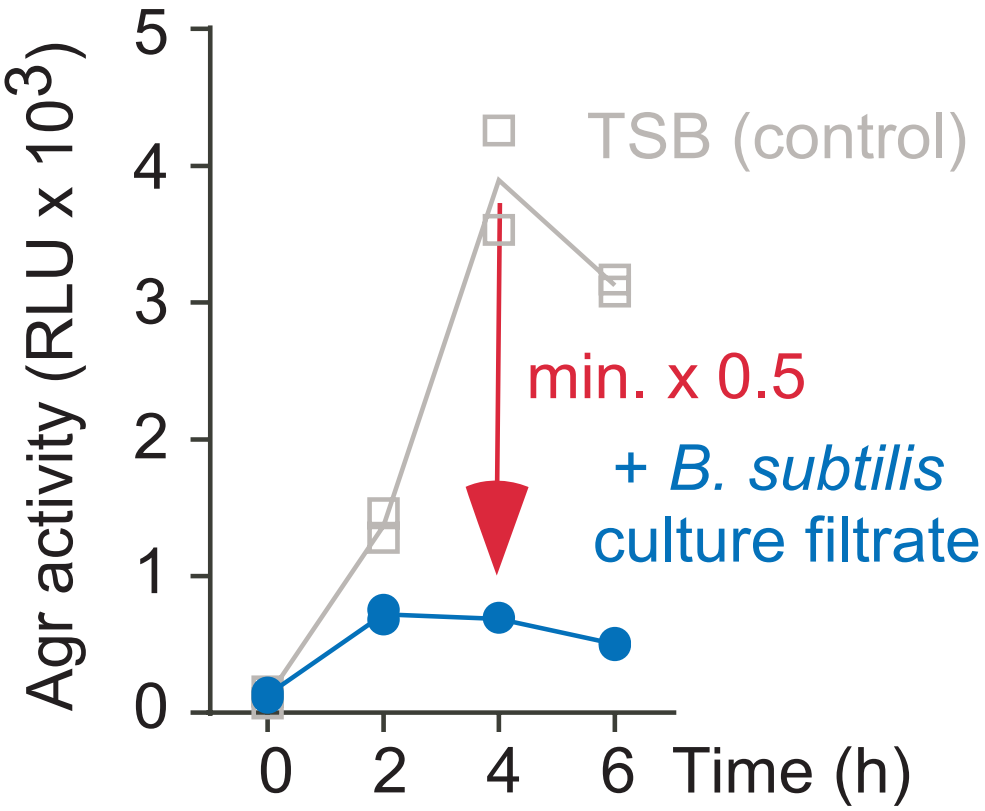
Non-competitive experiment with genetically complemented strains



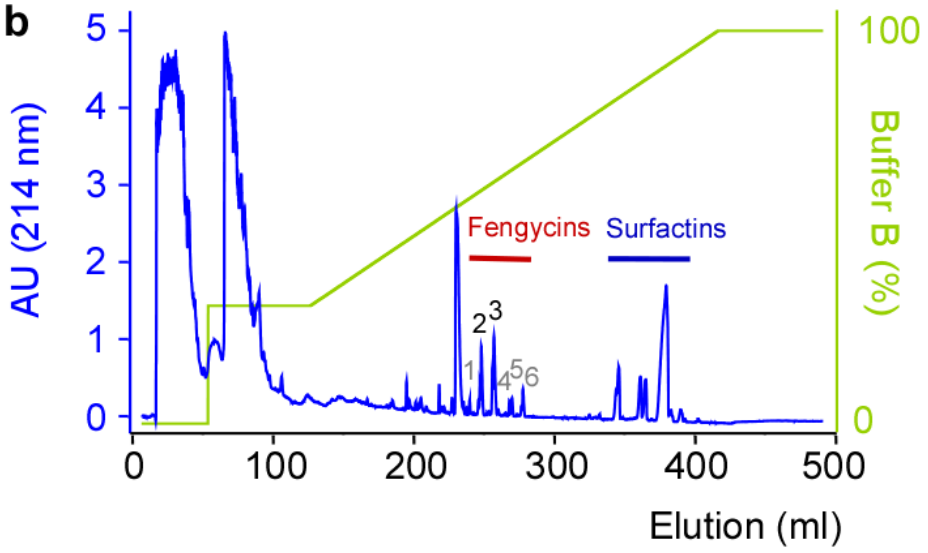
The Agr quorum-sensing system is indispensable for intestinal colonization.

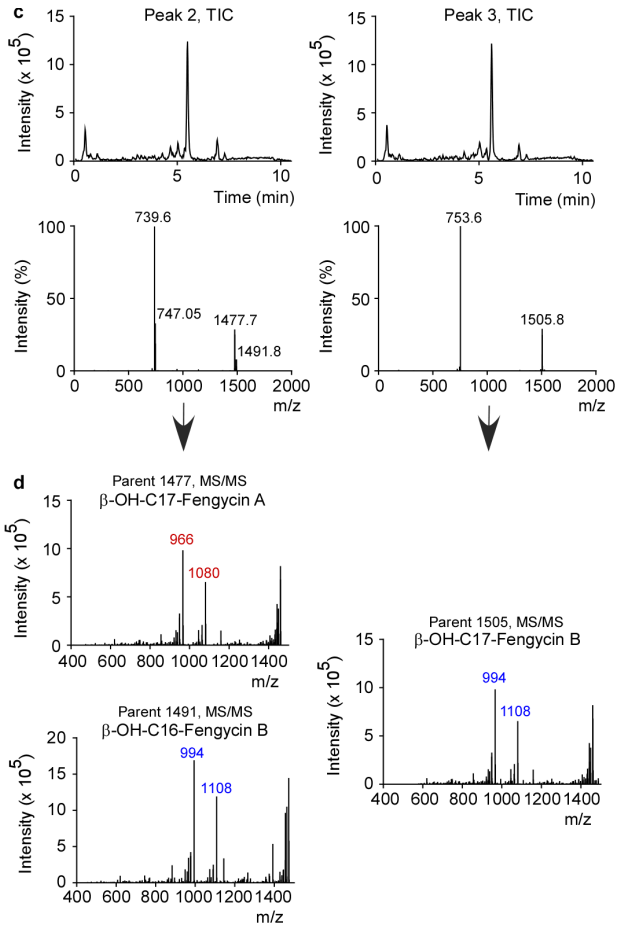
Agr inhibition by *Bacillus* culture filtrates

AgrP3-lux reporter



Identification of active molecules





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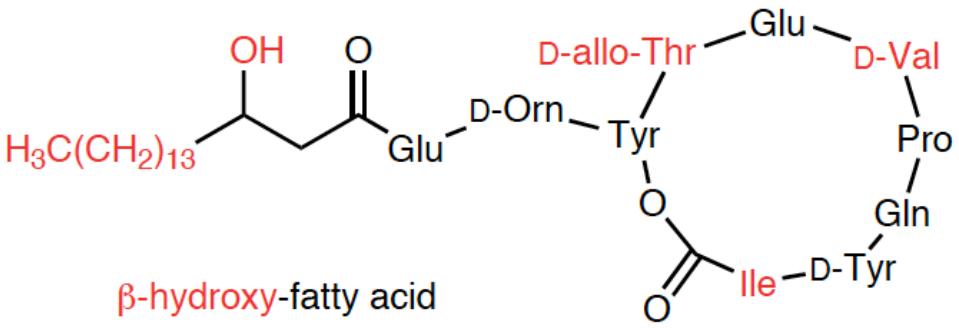
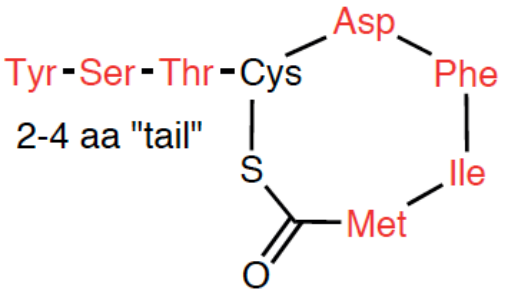
Molecular structure of Fengycins and AIP



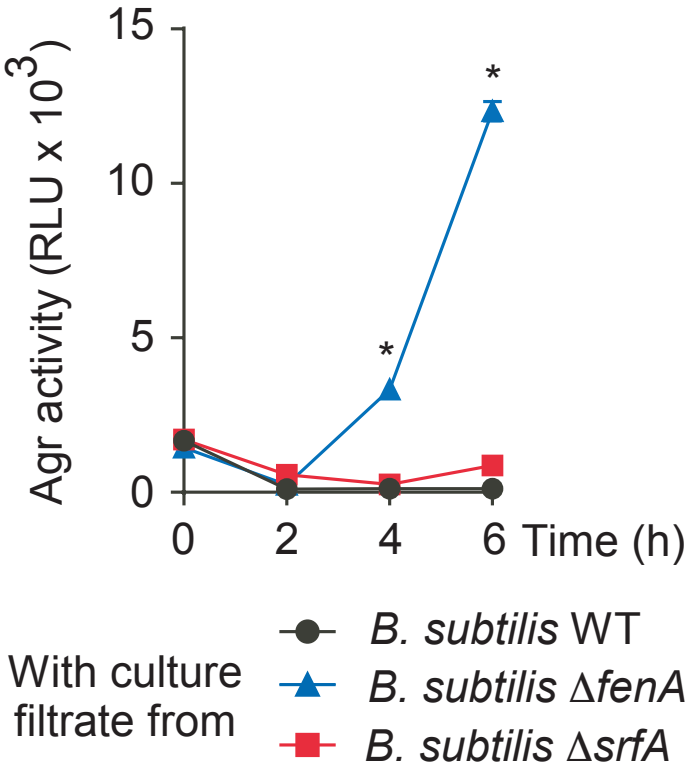
β-OH-C17-Fengycin B



S. aureus AIP I

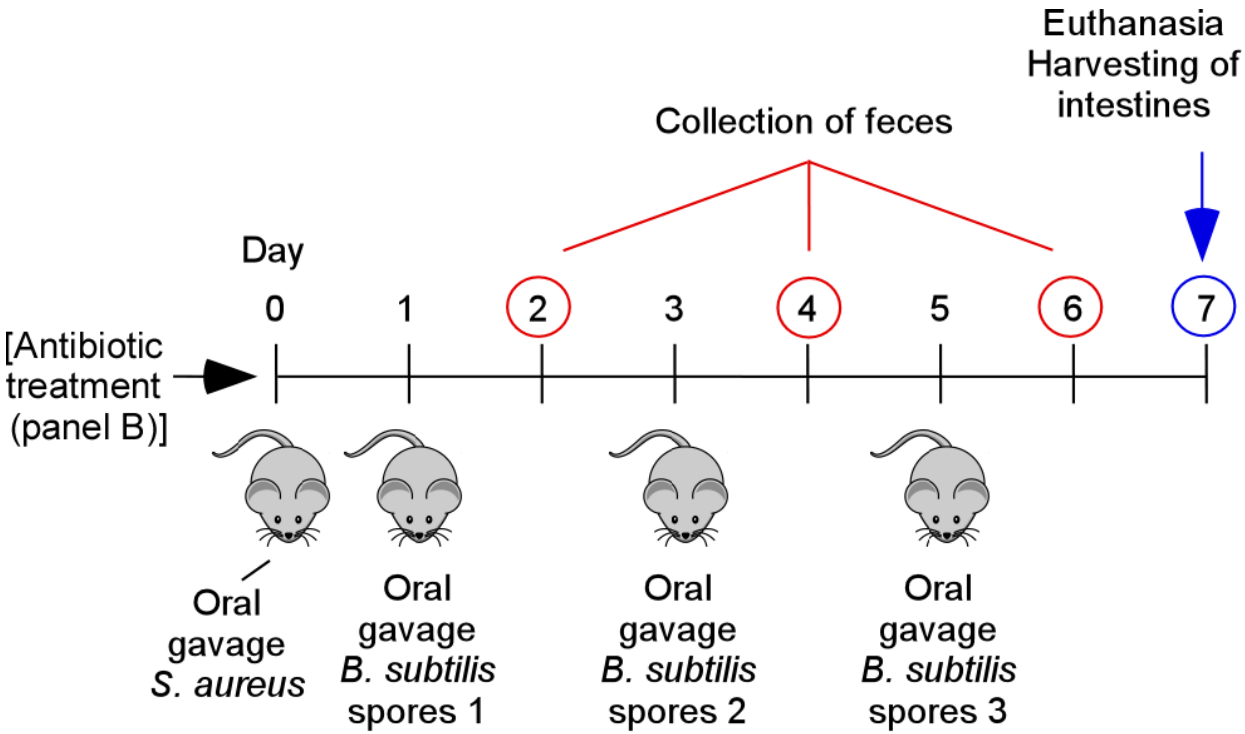
=> Structural similarity suggests mechanism of competitive inhibition.

Fengycin specificity – use of *fenA* mutant

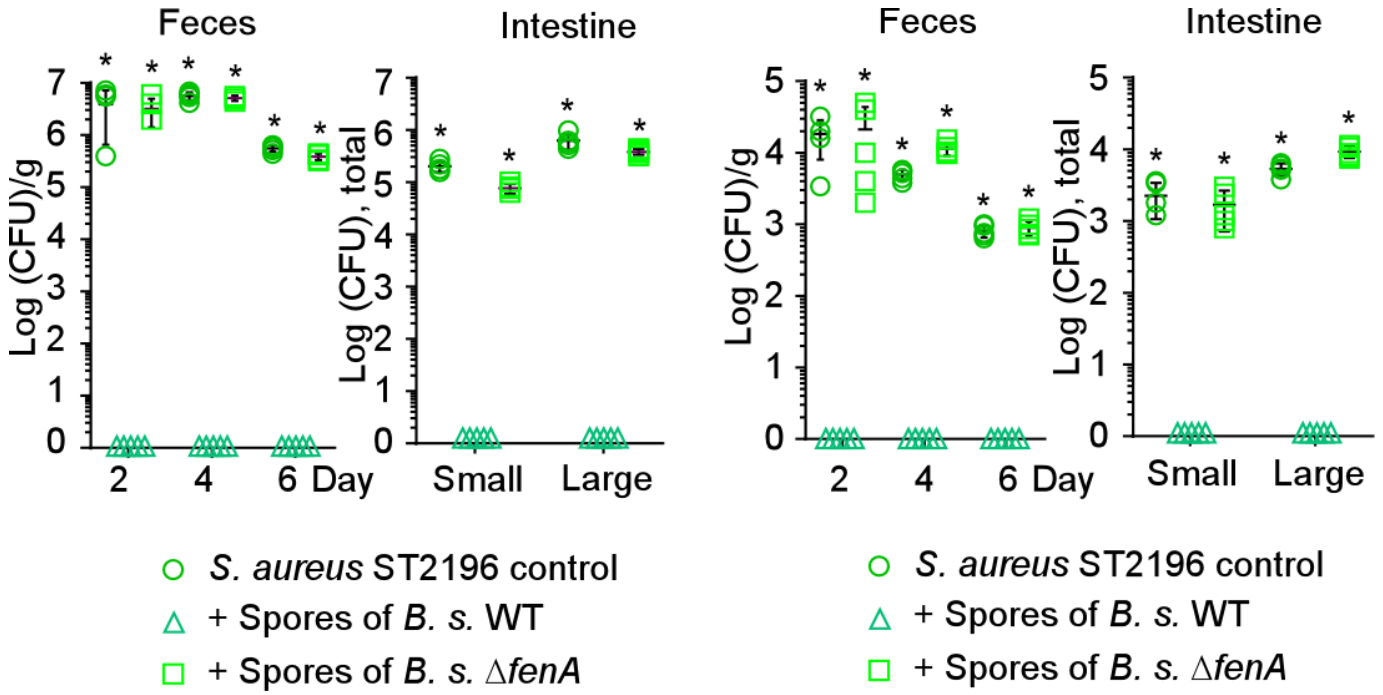


=> Fengycins are responsible for Agr inhibitory effect.

Setup of mouse experiment

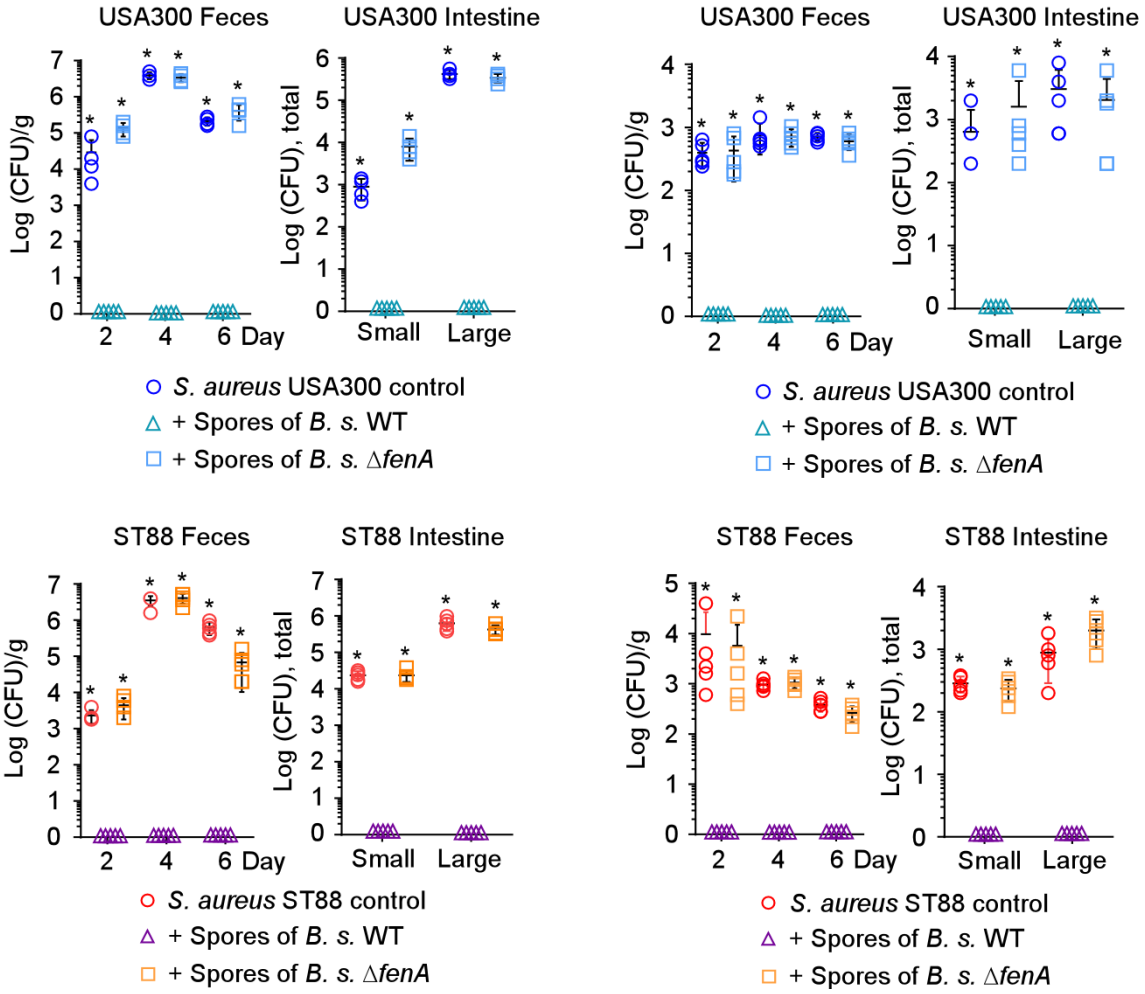


Inhibition of *S. aureus* colonization by fengycin-producing *Bacillus* in a mouse model



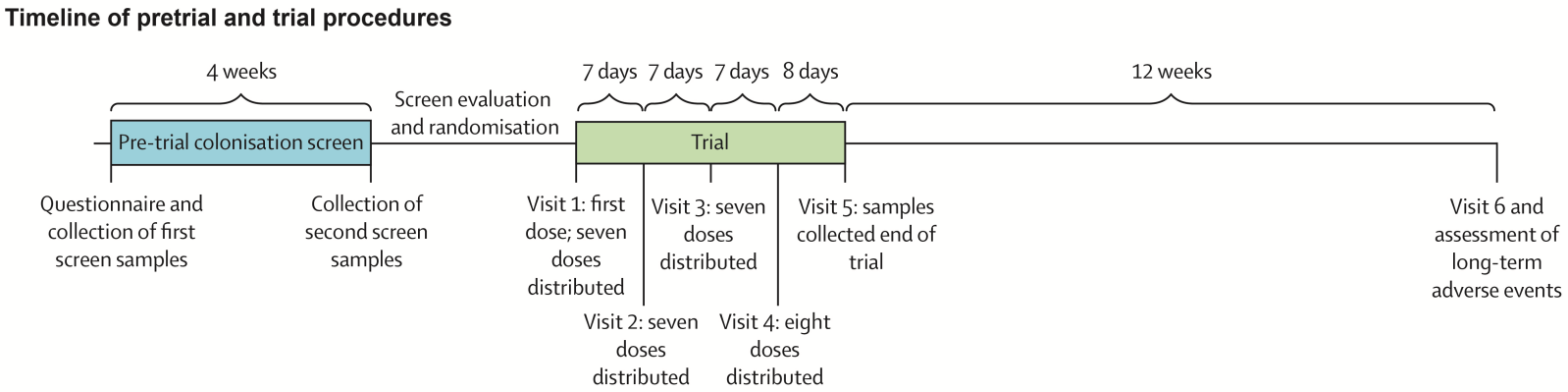
Oral application of *B. subtilis* wild-type, but not *fenA* mutant spores eliminated *S. aureus* colonization in the feces, small intestine, and large intestine.

Inhibition of *S. aureus* colonization by fengycin-producing *Bacillus* in a mouse model



Can we control *S. aureus* colonization in humans by oral application of *B. subtilis* spores?

Human trial: setup



Each dose: 10 x 10^9 CFU *B. subtilis* spores (OPTI-BIOME, *B. subtilis* MB40)

Placebo: Maltodextrin

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Piewngam et al. *Lancet Microbe* 2023

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Adverse effects

	Probiotic (n=55)	Placebo (n=60)	p value
Fever	0	0	1·00
Infection	0	0	1·00
Nausea and vomiting	4 (7%)	4 (7%)	1·00
Constipation	3 (5%)	2 (3%)	0·67
Headache	0	0	1·00
Muscle pain, cramp, or spasm	0	0	1·00
Upset stomach or heartburn	3 (5%)	2 (3%)	0·67
Gas or bloating	0	2 (3%)	0·50
Unusual stool (loose, discoloured, or more frequent)	3 (5%)	2 (3%)	0·67
Bad taste	4 (7%)	1 (2%)	0·19

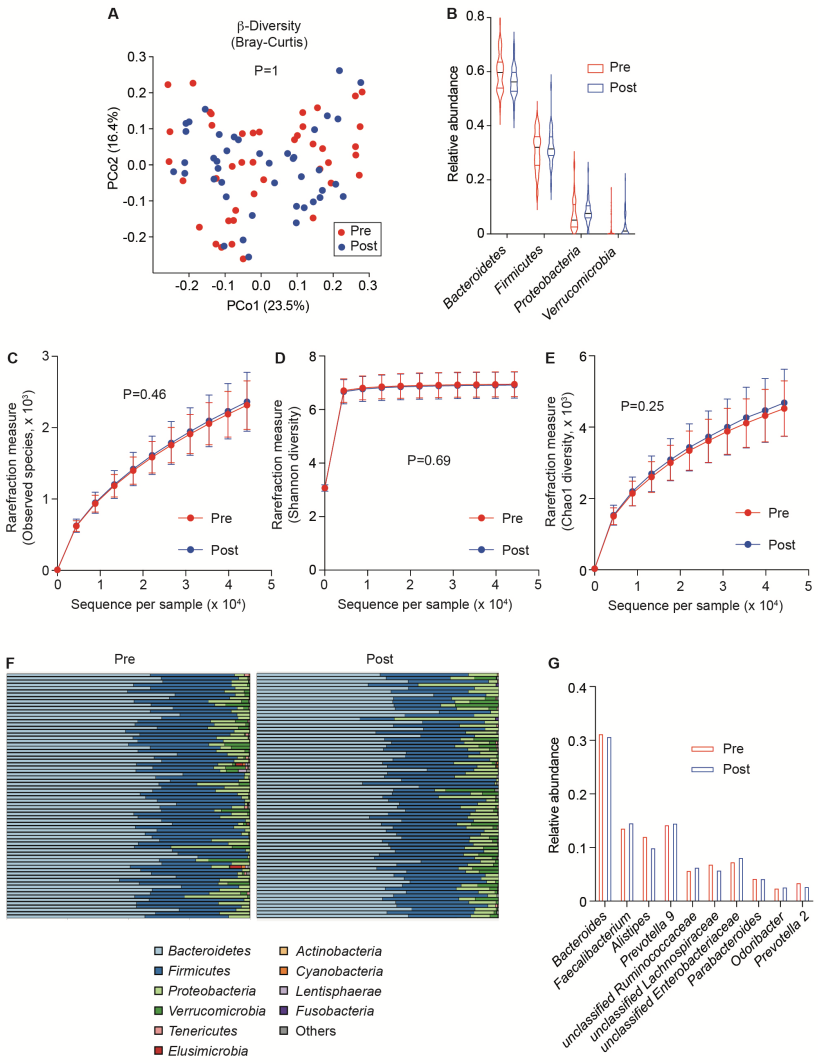
p value was established using two-tailed Fisher's exact test.

No severe adverse effects
No adverse effects significantly more frequent than in placebo group

Microbiome analysis feces before vs. after treatment

No differences (phylum and genus level)

Note:
Even bacteriocins are never that specific!



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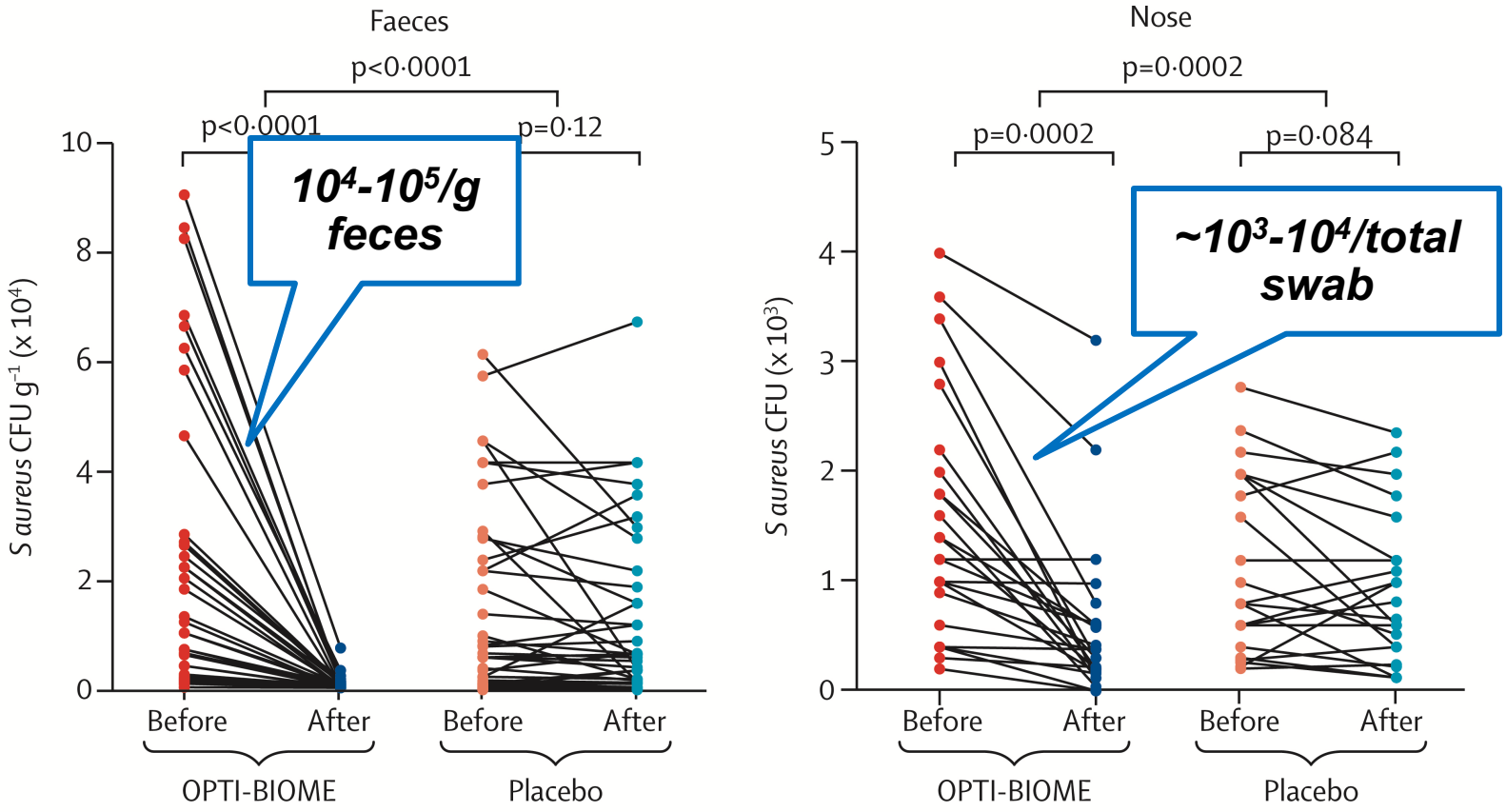
Trial results



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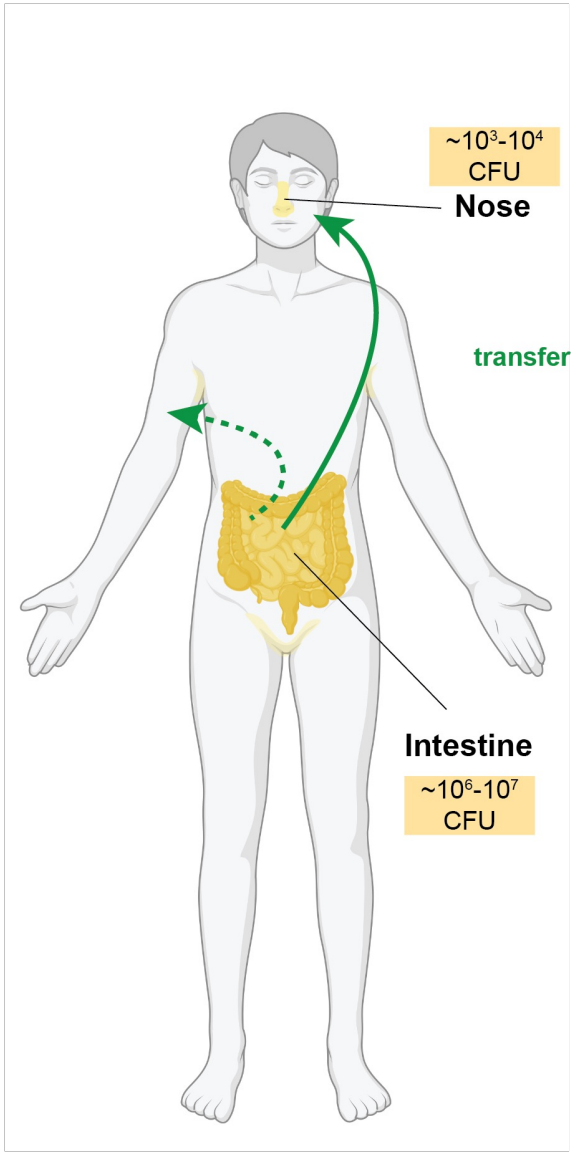
S. aureus colonization sites in quantitative terms

Even by a conservative estimate, intestinal *S. aureus* numbers exceed those in the nose (and minor other sites) by at least 2 logs.

***B. subtilis* spore treatment decreased total *S. aureus* CFU in the human body by > 95%.**

Results indicate dominant role of intestinal colonization site and transfer from there to the nose.



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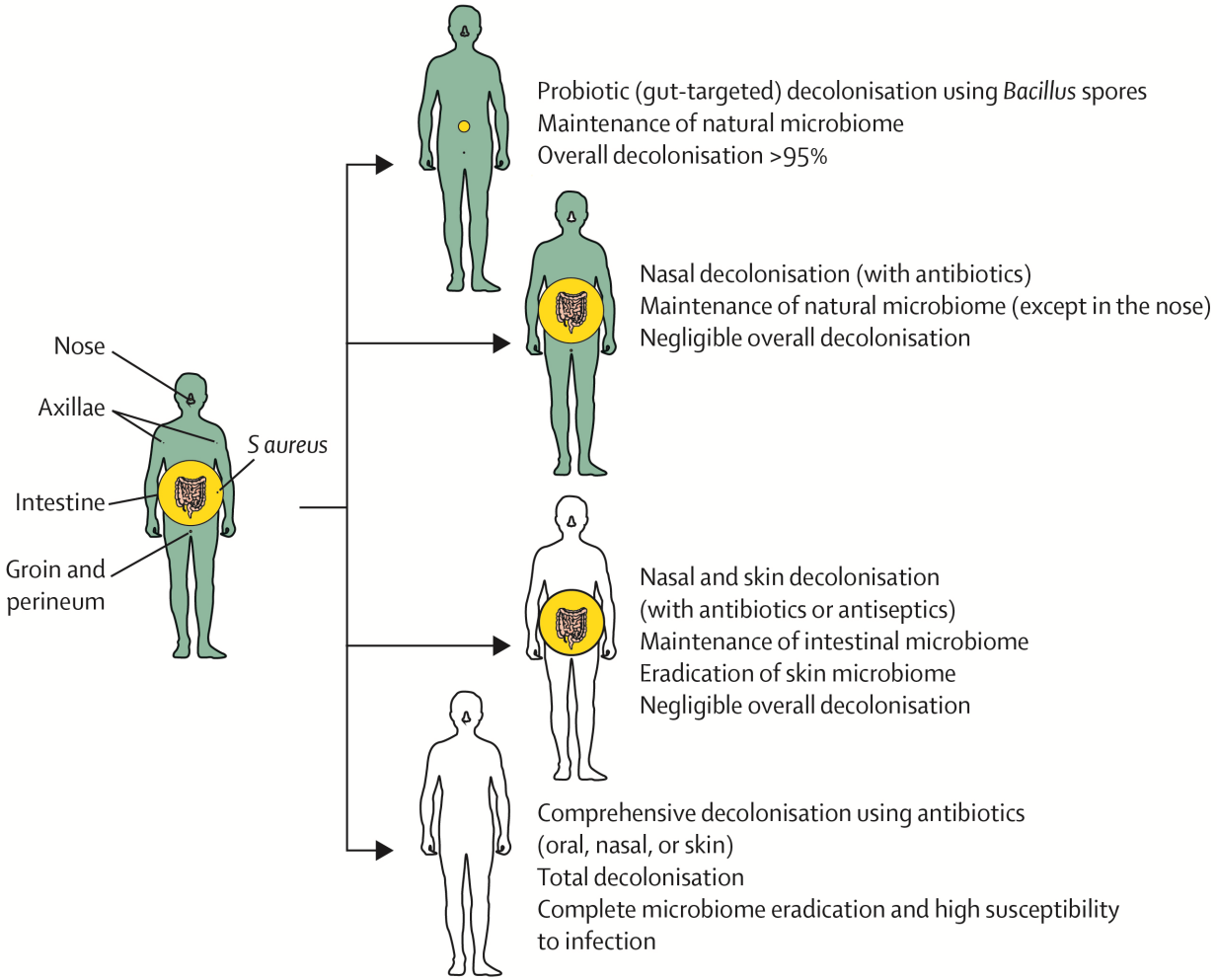
Comparison of decolonization strategies



Conclusions

- Agr controls intestinal colonization
- *B. subtilis* fengycins inhibit Agr.
- *B. subtilis* spores may used to decolonize *S. aureus* from the human body.
- Trials whether this leads to reduction of *S. aureus* infections (e.g., recurring skin infections) are planned
- Our results also indicate a more pivotal role of the intestine for overall *S. aureus* colonization in humans than previously assumed.

Thanks to…

NIAID/Otto group

Pipat Piewngam
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Thailand/Rajamangala
University of Technology Srivijaya

Sunisa Khongthong

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Natthrit Roekngam
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Damrongsak Faroongsarng

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